In the current study, we have checked the impact of the oxidized heme (hemin) on mitochondrial membrane potential and respiration rate of rat brain mitochondria. We have shown that hemin prevents the collapse of membrane potential that is normally caused by calcium-dependent BK channel openers (NS1619). A similar, though rather modest effect was observed in studies of oxygen consumption rate. We also report inhibitory effects of hemin on the reactive oxygen species-downregulating properties of NS1619.

Additionally, we have studied the single channel activity of mitoBK$_{Ca}$ by patch-clamp of mitoplasts isolated from a rat astrocyte cell line. Other results confirm the phenomenon of reversible inhibition of mitoBK$_{Ca}$ channel by hemin.

This might explain some of the cytotoxic effects of hemin observed in hemorrhagic stroke.

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11P2

The BK$_{Ca}$ channel is present in mitochondria of endothelial cell

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Large conductance Ca^{2+}-regulated potassium channels (BK$_{Ca}$, MaxiK, *Slo*1) are expressed in plasma membrane of different cells. They are involved in many processes such a signaling, neuronal excitability, vascular tone regulation, and neurotransmitter release. Also, BK$_{Ca}$ channels have been found in the inner mitochondrial membrane. It is believed that potassium channels regulate the mitochondrial membrane potential, matrix volume, respiration, and Ca^{2+} ion homeostasis. There are also hypotheses that mitochondrial potassium channels participate in neurodegenerative disorders and ischemic preconditioning.

In our study a single channel activity was measured after patch-clamp of the mitoplasts isolated from endothelial cell line (EA.hy 926). A potassium selective current was recorded with mean conductance 270 ± 10 pS in symmetrical 150 mM KCl solution. The channel was regulated by calcium and activated by NS1619, an activator of BK$_{Ca}$ channel. In opposite, activity of the channel was blocked irreversibly by paxilline and iberiotoxin, inhibitors of BK$_{Ca}$ channel. Also, inhibitors of mitochondrial ATP-regulated potassium channel (ATP/Mg^{2+}, 5-HD, glibenclamide) were tested and no effects on observed activity of ion channel were detected. Taken together, our findings indicate that mitochondrial large conductance Ca^{2+}-regulated potassium channels with properties similar to the surface membrane BK$_{Ca}$ channel are present in endothelial mitochondria.

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11P3

Histones destabilize the mitochondrial membrane organization and cause release of cytochrome *c*

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Extensive DNA damage leads to apoptosis. Histones play a central role in DNA damage sensing and they may mediate signals of genotoxic damage to cytosolic effectors including mitochondria [1]. In this study we have investigated the effects of histones on mitochondrial function and membrane integrity. Both linker histone H1 and the core histones H2A, H2B, H3, and H4 bind strongly to isolated mitochondria [2]. The binding resulted in a rapid and massive release of the pro-apoptotic intermembrane space proteins cytochrome *c* and Smac/Diablo indicating that histones permeabilize the outer mitochondrial membrane. In addition, linker histone H1, but not core histones, induced a collapse of the mitochondrial membrane potential, release of pyridine nucleotides, and mitochondrial fragmentation. We conclude that both linker and core histones destabilize the mitochondrial membranes and we postulate that this mechanism may boost apoptosis signaling following DNA damage.

References

[1] A. Konishi, S. Shimizu, J. Hirota, T. Takao, Y. Fan, Y. Matsuoka, L. Zhang, Y. Yoneda, Y. Fujii, A. Skoultchi, Y. Tsujimoto, Involvement of histone H1.2 in apoptosis induced by DNA double-strand breaks, Cell 114 (2003) 673–688.
[2] A. Cascone, C. Bruelle, D. Lindholm, P. Bernardi, O. Eriksson, Destabilization of the outer and inner mitochondrial membranes by core and linker histones, PLoS ONE 7 (2012) e35357.

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11P4

Mitochondrial calcium-independent phospholipase iPLA2γ is directly activated by H_2O_2 *in vitro*

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Calcium-independent phospholipases (iPLA2s) are a family of enzymes that are known to participate in cellular signaling by simultaneously producing free fatty acids and lysophospholipids. Mitochondria contain predominantly iPLA2γ and we have shown previously that the activity of mitochondrial iPLA2γ (mt-iPLA2γ) is increased following the addition of *tert*-butyl hydroperoxide (TBHP) or H_2O_2, thus indicating a redox-sensitive process [1]. Here we tested the hypothesis that mt-iPLA2γ is activated by H_2O_2 directly. Using isolated mouse lung mitochondria, we followed the changes in mitochondrial respiration and membrane potential. Mt-iPLA2γ-dependent TBHP or H_2O_2-induced uncoupling of oxidative phosphorylation was not inhibited by staurosporine, a broad-spectrum inhibitor of protein kinases, or by chelerythrine, an inhibitor of protein kinases C, which indicates that redox-activated kinase cascade pathways are not involved in this iPLA2γ-dependent event. Using human recombinant iPLA2γ reconstituted into liposomes, we followed fluorometrically proton transport across the liposomal membranes. The addition of H_2O_2 caused intraliposomal acidification, indicating H_2O_2-induced iPLA2γ-dependent release of free fatty acids. This effect was inhibited by (R)-bromoenol lactone (BEL), a selective inhibitor of iPLA2γ, but not by its optical enantiomer (S)-BEL. Using GC–MS chromatography, we detected directly the amount of free fatty acids following each experimental protocol. Our results are consistent with